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Review of Previous Records**

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Source: Comparative Parasitology, 79(1):30-43. 2012.

Published By: The Helminthological Society of Washington

DOI: <http://dx.doi.org/10.1654/4493.1>

URL: <http://www.bioone.org/doi/full/10.1654/4493.1>

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Parasites and Pathogens of Eastern Bluebirds (*Sialia sialis*): A Field Survey of a Population Nesting Within a Grass-Dominated Agricultural Habitat in Georgia, U.S.A., with a Review of Previous Records

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ABSTRACT: The parasite community associated with a population of eastern bluebirds (*Sialia sialis*) nesting in a grass-dominated habitat in northern Georgia, U.S.A., was surveyed by live examination of adults and nestlings; examination of feces, nesting materials, and blood smears; necropsy; and polymerase chain reaction techniques. During the course of 5 breeding seasons, we found 10 macroparasite species, which included 1 tick species (*Ixodes brunneus*), 1 louse species (*Philopterus sialii*), 1 species of hematophagous mite (*Dermanyssus prognephilus*), and 5 other species not previously reported from eastern bluebirds, including an acanthocephalan (*Plagiorhynchus cylindraceus*), a species of stronglyloid nematode, a cestode (*Hymenolepis* sp.), a nestling bird fly (*Carnus floridensis*), and a nest mite (*Campephiloptes* sp.); a description of feather mites associated with the population was not included in this report. Overall prevalence of macroparasites was 16.1%. We also detected 5 species of protistan parasites, including *Haemoproteus fallisi*, *Plasmodium relictum*, *Trypanosoma avium*, and 2 others, an *Atoxoplasma* sp. and a *Sarcocystis* sp., which represent new host–parasite records. One bird tested positive for *West Nile virus* and for *St. Louis encephalitis virus*. More than 50% of the birds examined were infected with 1 or more microparasites. Prevalence of microparasites varied by examination method employed. An updated list of known parasites of eastern bluebirds gathered from published literature and database searches is provided.

KEY WORDS: Acanthocephala, *Atoxoplasma*, *Carnus floridensis*, *Haemoproteus*, nematode, PCR, *Plagiorhynchus cylindraceus*, *Plasmodium*, *Sarcocystis*.

Eastern bluebirds, *Sialia sialis*, are one of the most readily recognized North American songbird species because of their wide distribution, bright coloration, relative tameness, and ready acceptance of nest boxes (Gowaty and Plissner, 1998). No evidence suggests that infectious disease agents play a role in population regulation; however, information on parasites affecting bluebirds is limited (Gowaty and Plissner, 1998). Current knowledge of parasites and disease in bluebirds is based on the occasional opportunistic encounter with dead, injured, or sick birds; observational studies of nesting adults and nestlings; and collections of parasites during surveys that are not species specific. Although Chow et al. (1983) did a limited study of parasites and other invertebrates from 13 eastern bluebird nests and Hicks (1959) extensively reviewed older literature on bluebird nest inhabitants, including several parasitic forms, only Roberts (1981) has previously summarized in one

place many of the parasites known to infest eastern bluebirds.

The objectives of this study were to collect and identify parasites and pathogens associated with a population of eastern bluebirds nesting within a grass-dominated, agricultural habitat in northern Georgia, U.S.A. In addition, given that the Roberts (1981) review is now 30 yr old and was based on literature published between 1936 and 1977, we sought to update the list of known parasites associated with eastern bluebirds to provide a more modern perspective for our results.

MATERIALS AND METHODS

We studied eastern bluebirds over 5 breeding seasons (March–August, 2004–2006, 2008, and 2009) within a 11,331-ha college campus land tract located in Floyd County, approximately 104 km northwest of Atlanta, Georgia, U.S.A. (34°16′58.0758″N; 85°11′30.4902″W). Roughly 1,200 ha were suitable bluebird habitat, divided among 3 noncontiguous areas of active agricultural usage. Each area had habitat characteristics required by bluebirds, namely, open areas of primarily grassy vegetation, perching

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sites used to locate prey, and structures supporting nesting sites (Gowaty and Plissner, 1998).

We captured breeding adult birds inside nest boxes using a trap door system. Each bird was examined for ectoparasites by gently ruffling through its feathers and exposing featherless tracts. To minimize stress and reduce the risk of nest abandonment, birds were released within 8 min of capture. Ectoparasites, when detected, were placed into a suitably labeled vial containing 70% ethanol (ETOH) for pending identification.

We collected fecal material during capture occasions and held it at room temperature for 24 hr to allow for sporulation of oocysts. Two drops of 10% buffered formalin were added to each sample as a preservative before storage at 4°C. Samples were prepared for centrifugation and flotation with Sheather's sugar solution (Georgi and Georgi, 1990), and a sedimentation technique (Truant et al., 1981), with CitriSolv (Fisher Scientific, South Bend, Indiana, U.S.A.) as the solvent, was used to further study fecal samples collected in 2008 and 2009. All samples were examined at $\times 100$ magnification with a compound light microscope and were denoted as positive if 1 or more parasite ova or oocysts were detected per sample. We (R.E.C. and M.J.Y.) identified ova to the level of family or genus, as possible, matching morphologic descriptions and measurements as closely as possible to descriptions and measurements found in related literature (Read, 1949; Kassai, 1999; Atkinson et al., 2008).

Blood samples were obtained from adult bluebirds by right jugular venipuncture. Blood smears were air-dried and stained with Hemacolor® Staining Solution (EM Science, Gibbstown, New Jersey, U.S.A.) after fixation and examined at $\times 1,000$ under oil immersion on a compound light microscope for presence of hematozoa. Hematozoa detected in this way were identified (by R.E.C.) using morphologic characteristics defined by previously identified samples from this same host population (Carleton et al., 2004).

Because safety concerns limited more extensive sampling on living birds, during the final weeks of the 2006 breeding season, 14 males were killed by either exsanguination or carbon dioxide intoxication and necropsied. Bluebird offspring survival is not significantly reduced following loss of the male from a breeding pair (Gowaty, 1983), and therefore, we anticipated no, or minimal, negative effects on the local population. Each carcass was immediately placed in a sealable plastic bag and refrigerated until necropsy to prevent ectoparasites from escaping. Necropsies were completed between 4 and 16 hr of collection, following standard protocol (van Riper and van Riper, 1980). Nasal passages were flushed by forcing a water and ETOH solution through the choanal opening. Wash solution was captured in a petri dish and examined under a dissecting microscope at $\times 40$ magnification. After sealing the pharyngeal area and nares with cotton, each carcass was washed with a water-isopropyl alcohol-soap solution and was then rinsed thoroughly (Clayton and Walther, 1997). Ectoparasites were separated from the wash solution by vacuum filtration and placed into labeled vials containing 70% ETOH. We followed the techniques of Doster and Goater (1997) to detect, collect, and quantify endoparasites. Fecal material present in the large intestine was collected and examined as previously described.

To screen the population for *West Nile virus* and *St. Louis encephalitis virus*, we submitted 2 ml of serum from the 14 adult birds collected for necropsy to the Southeastern

Cooperative Wildlife Disease Study (SCWDS) in Athens, Georgia, U.S.A., for testing by plaque reduction neutralization.

We extracted DNA from each of the 14 adult bluebirds using approximately 5 mg of homogenized spleen tissue obtained during necropsy and the GFX Genomic Purification Kit (Amersham Pharmacia Biotech, Piscataway, New Jersey, U.S.A.) according to the manufacturer's instructions. A nested polymerase chain reaction (PCR) was used to amplify a portion of the 18S ribosomal RNA (rRNA) gene common to all species of *Eimeria*, *Isospora*, and *Atoxoplasma*. The primary PCR-amplification substrate consisted of 10 μ l of DNA in a 25- μ l reaction containing 10 mM Tris-chloride (pH 8.3), 50 mM potassium chloride, 1.5 mM magnesium chloride, 0.2 mM each deoxynucleoside triphosphate, 2.5 units Taq DNA Polymerase (Promega Corp., Madison, Wisconsin, U.S.A.), and 25 pmol of primers EIMF (5'-ACCATGGTAATCTATG-3') and 990 (5'-TTGCC-TYAAACTTCCTT-3'). Secondary PCR amplification was identical, except that the primers were EIMR (5'-CTCAAAGTAAAAGTTCC-3') and 989 (5'-AGTTTCT-GACCTATCAG-3') (Yabsley and Gibbs, 2006). Reaction conditions consisted of 30 cycles of the following temperature regime: 94°C for 30 sec, 40°C for 1 min, and 72°C for 1 min plus 1 sec/cycle, followed by a final elongation of 72°C for 12 min. To detect additional protist DNA in the splenic tissue, we used a nested PCR to amplify a portion of the cytochrome b genes of *Haemoproteus* and *Plasmodium*; primers HAENF and HAEMNR2 were used in a primary reaction and HAEMF and HAEMR2 in a secondary reaction, as described by Waldenström et al. (2004). Amplified products were separated in 2% agarose gels, stained with ethidium bromide, and visualized with ultraviolet light. Products were purified with a Microcon spin filter (Amicon Inc., Beverly, Massachusetts, U.S.A.) and were sequenced at the University of Georgia Integrated Biotech Laboratories (Athens, Georgia, U.S.A.).

We collected representative sections of skeletal muscle with suspected sarcocysts from each of 3 necropsied adult birds into 10% buffered formalin for histopathology and froze other samples from them at -20°C for PCR analysis. Histologic sections were cut at 4 μ m, stained with hematoxylin and eosin, and examined at $\times 400$ magnification. A portion of the 18S rRNA gene was amplified by nested PCR as described and using primers 18S9L and 18S1H (Li et al., 2002). Amplicons were purified and sequenced as described previously.

During the course of the study, we collected 5 dead, adult bluebirds (2 females and 3 males) in the field. A minimal state of autolysis allowed partial necropsy of these individuals. We did not process these birds by body wash because of probable ectoparasite abandonment of the carcasses but did examine the integument, musculoskeletal system, gastrointestinal tract, liver, and heart.

We examined 10-d-old nestlings for ectoparasites and skin lesions suggestive of parasite-induced injury. Representative samples of ectoparasites, if present, were collected from 1 or more nestlings within each clutch and placed into labeled vials containing 70% ETOH.

After a brood fledged or was depredated, we removed old nesting material from all boxes but saved several nests for examination. These were sealed in plastic bags and stored under refrigeration until examination. Because of excessive amounts of feather dust and other fine debris within the

Table 1. Methods used to detect parasites and pathogens from an eastern bluebird (*Sialia sialis*) population in Georgia, U.S.A., from 2004 to 2006, 2008, and 2009 and the numbers of birds examined by each method.

Parameter	No.	Comment
Live examination of adults	230	Visual examination for ectoparasites by ruffling feathers and exposing pterygiae. Birds were released following examination.
Live examination of clutches of nestlings	103	Body and integument of 10-d-old nestlings was visually examined for ectoparasites. Nestlings were returned to their nest boxes following examination.
Examination of nesting materials	103	Nests were collected and placed in sealed plastic bags after the nest box was vacated. Nesting materials were inspected for arthropods visible to the naked eye.
Complete necropsy	14	Birds were humanely killed, placed in sealed bags, and chilled. Necropsy consisted of an external examination, nasal flush, body wash in an alcohol solution, and internal examination, which included opening and examining the digestive tract, respiratory tree, and heart and collection of spleen tissue.
Partial necropsy	5	Birds found dead in the field were placed in sealed bags and chilled. Necropsy consisted of an external and internal examination, which included opening and examining the digestive tract and organs in a minimal state of autolysis.
Blood smear examination	135	Blood was collected via jugular venipuncture from captured adults and before complete necropsy. Smears were air-dried, fixed, stained with Hemacolor 3, and examined at $\times 1,000$ magnification for hematozoa.
DNA extraction via polymerase chain reaction technique	14	Spleen tissue or muscle tissue was tested for parasite DNA using standardized procedures.
Fecal flotation	189	Feces collected from adult birds were prepared using Sheather's Sugar solution, centrifugation, and flotation then examined at $\times 100$ magnification for parasite ova.
Fecal sedimentation	62	Feces collected from a subsample of adult birds were prepared by sedimentation then examined at $\times 100$ magnification for parasite ova.
Plaque reduction neutralization test	14	Serum was collected from live birds before complete necropsy and tested for antibodies to West Nile and St. Louis encephalitis viruses.

nesting material, a 70% ETOH solution was added to each bag to wet the contents. The material was then placed into a shallow pan and examined for arthropods that could be seen with the naked eye. The alcohol solution was decanted following disposal of nesting material and filtered for collection of microscopic arthropods. All ectoparasites collected from nests were placed in labeled vials containing 70% ETOH. Ectoparasites from these samples and from those collected from adult birds were examined and identified using standard methods and references at the Proctor Laboratory of Acarology, University of Alberta, Edmonton, Alberta, Canada, and the Parasitology Unit, Pathobiology Laboratory, National Veterinary Services Laboratories, United States Department of Agriculture (NVSL, USDA), Ames, Iowa, U.S.A.

RESULTS

A total of 249 eastern bluebird adults were examined by one or a combination of methods between 2004 and 2009, and 103 clutches of nestlings and nests were examined in 2004 (Table 1). Representatives of 8 species of macroparasites, excluding feather mites, were collected from among 44 (17.7% of 249) adult birds within the study population, including a cestode, an acanthocephalan, 3 species of nematodes, 1 tick, and 1 species of chewing louse.

Nearly all adult birds examined harbored 2 to 4 species of feather mites that are documented elsewhere (see Carleton and Proctor, 2010). We detected representatives of 5 species of protists among 104 (77.0% of 135) adult birds from which we examined blood smears or tested tissues for parasite DNA.

Hematophagous nest mites (*Dermanyssus prognephilus*) infested nestlings (43.7% of broods) from all sites, and all came from nests in which mites were present during nest examination. One clutch was infested with a nestling bird fly (*Carnus floridensis*). We also collected a possibly new species of nest mite (*Campephilocoptes* sp.).

West Nile virus and St. Louis encephalitis virus

Prevalence: One of 14 adult birds (7.1%) tested positive for both viruses by plaque-reduction neutralization testing.

Remarks: There are numerous previous reports of West Nile virus infection in eastern bluebirds. The virus was first isolated from Georgia (U.S.A.) birds in 2001 (Gibbs et al., 2006).

Apicomplexa
***Atoxoplasma* sp.**
Garnham, 1950

Prevalence: Positive results were returned from 7 of 14 necropsied, adult bluebirds (50.0%) examined by PCR technique.

Site of infection: Spleen.

Remarks: This finding represents a new host record for *Atoxoplasma* sp. Our sequence analysis of products produced by the *Eimeria/Isospora/Atoxoplasma* reaction was 98% identical to the only *Atoxoplasma* sp. sequence published in GenBank (GenBank AY331571). This sequence was obtained from a southern cape sparrow (*Passer melanurus melanurus*). All sequences of *Atoxoplasma* sp. from positive bluebirds were identical to each other. *Atoxoplasma* spp. have been reported in canaries (*Serinus canaria*) (Sánchez-Cordón et al., 2007), European greenfinches (*Carduelis chloris*) (Cooper et al., 1989), Eurasian bullfinches (*Pyrrhula pyrrhula*) (McNamee et al., 1995), Bali mynahs (*Leucopsar rothschildi*) (Partington et al., 1989), and 14 species of tanagers (Adkesson et al., 2005).

Haemoproteus fallisi
Bennett and Campbell, 1972

Prevalence: Seventy-nine of 135 blood smears (59.0%) from sampled, live, adult birds were positive. The mean intensity of infection was estimated at 3.4 (± 6.2 SD) infected erythrocytes per 10,000 erythrocytes. Spleen tissue from 1 of 14 adult bluebirds (7.1%) examined by PCR returned positive results.

Site of infection: Blood and spleen.

Specimens deposited (blood smear): U.S. National Parasite Collection (USNPC 102318.00).

Remarks: Identification was based on morphologic characteristics of gametocytes in blood smears and earlier known associations of the species with other members of the avian subfamily Turdinae (Greiner et al., 1975; Bennett and Pierce, 1988).

The single *Haemoproteus* infection detected by PCR was 98% identical to a *Haemoproteus* sp. haplotype 2 (GenBank AF465563) obtained from both a yellow-throated warbler (*Dendroica dominica*) and a northern parula (*Parula americana*). This sequence was also 98% identical to a *Haemoproteus* sp. haplotype 1 (GenBank AF465562) obtained from

both an eastern bluebird and a western bluebird (*Sialia mexicana*).

Plasmodium relictum
Grassi and Feletti, 1891

Prevalence: Ten of 14 adult bluebirds (71.4%) examined using PCR had evidence of infection.

Site of infection: Spleen.

Remarks: Although *Plasmodium* sp. has been previously reported in eastern bluebirds (Table 2), *P. relictum* has not. Sequences from 9 (90%) of the *Plasmodium*-infected birds were 99% identical to a *P. relictum* (GenBank AY733088) sample obtained from a captive jackass penguin (*Spheniscus demersus*). The sequence from the remaining bluebird had several polymorphic bases but was 98% identical to *P. relictum*. No infections with *Plasmodium* spp. were detected in blood smears, including smears from the positive birds.

***Sarcocystis* sp.**
Miescher, 1843

Prevalence: Three of 19 necropsied, adult bluebirds (15.8%) harbored sarcocysts.

Site of infection: Skeletal muscle of all 3 birds (100%) and connective tissue associated with the caudal surface of the left eye of 1 bird.

Specimens deposited: A slide of muscle tissue containing cysts was deposited in the U.S. National Parasite Collection (USNPC 102319.00).

Remarks: This represents the first report of a *Sarcocystis* sp. from eastern bluebirds. Two birds had cysts within all major muscle groups, but the third had cysts in only wing, leg, and back muscles. We measured representative muscle cysts as 2.16×0.74 , 2.52×0.77 , and 1.84×0.77 mm. We also found cysts within connective tissue associated with the caudal surface of the eye of 1 bird harboring the largest number of cysts. No inflammation was associated with the muscle cysts.

Sequences of the partial 18S rRNA gene (792 base pair [bp]) of the *Sarcocystis* sp. from the 3 bluebirds were identical. The sequences were 100% identical (of the 359 available overlapping bases) to *S. falcatula* strain Stiles (GenBank AY628220) and also 100% identical (for 752 bp overlapping bases) to a *Sarcocystis* sp. AGP-1 (GenBank DQ768305) reported from a captive African grey parrot (*Psittacus erithacus*) (Dubey et al., 2006). The bluebird

Table 2. Microparasites previously detected in eastern bluebirds (*Sialia sialis*) based on literature reports (1941–2009).*

Species	Host locality†	Reference
Viruses		
Eastern equine encephalitis	FL	Favorite (1960)
	NY	Molaei et al. (2006)
West Nile virus	FL	Forrester and Spalding (2003)
	GA	Gibbs et al. (2006)
	NY	Bernard et al. (2001)
	ns	LaDeau et al. (2007)
Apicomplexa		
<i>Haemoproteus</i> sp.	ns	Ricklefs and Fallon (2002)
<i>H. fallisi</i>	ns	Greiner et al. (1975)
	GA	Mataxas and Pung (1999), Carleton et al. (2004)
<i>H. oryzivora</i>	ns	Greiner et al. (1975)
<i>Leucocytozoon</i> sp.	MD	Wetmore (1941), Williams and Bennett (1978)
<i>L. dubreuli</i>	ns	Greiner et al. (1975)
<i>L. majoris</i>	ns	Greiner et al. (1975)
<i>Plasmodium</i> sp.	MD	Wetmore (1941), Williams and Bennett (1978)
	NJ	Williams and Bennett (1978)
	ns	Greiner et al. (1975)
Euglenozoa		
<i>Trypanosoma</i> sp.	MD, NJ	Williams and Bennett (1978)
<i>T. avium</i>	MD	Wetmore (1941), Greiner et al. (1975)
	GA	Mataxas and Pung (1999), Carleton et al. (2004)

*FL, Florida; NY, New York; GA, Georgia; ns, not specified; MD, Maryland; NJ, New Jersey.

†Localities are within the United States unless specified otherwise.

Sarcocystis sp. was only 99.7% identical (790 of 792 bases) to *S. neurona* (SNU07812). Although our amplified genetic sequences matched the sequence on file for *S. falcatula*, the 18SrRNA gene is highly conserved in these protists (Dame et al., 1995), hindering definitive species determination within the group. Other genetic targets possibly could provide more specific identification information at some point (Tanhauser et al., 1999).

Euglenozoa
Trypanosoma avium
Danilewsky, 1885

Prevalence: Four of 135 blood smears (2.9%) from adult birds were infected.

Site of infection: Blood.

Specimens deposited: U.S. National Parasite Collection (USNPC 102320.00).

Remarks: The intensity of infection was minimal in each of the birds examined. We found only 1 parasite in 3 of the 4 positive blood smears and only 2 parasites in the fourth.

Acanthocephala
Plagiorhynchus cylindraceus
(Goeze, 1782) Schmidt and Kuntz, 1966

Prevalence: Eight of 19 necropsied, adult birds (42.1%) were infected. No fecal samples examined from necropsied birds contained ova. One of 62 fecal samples (1.6%) examined using the sedimentation technique contained ova. These ova matched morphologic descriptions consistent with *P. cylindraceus*.

Site of infection: Small intestine.

Specimens deposited: U.S. National Parasite Collection (USPNC 102321.00).

Remarks: *Plagiorhynchus cylindraceus* represents one of several new host–parasite records resulting from our study. This acanthocephalan was reported previously in western bluebirds (*Sialia mexicana*) and implicated as a contributory cause of death in a small number of cases (Thompson-Cowley et al., 1979; Bildfell et al., 2001). The 2 dead bluebirds found during our study harbored 6 and 8 *P. cylindraceus*, respectively. Three or fewer worms were found in the other necropsied birds. In both cases, all of the worms

were attached to the intestinal wall in proximity to each other and seemed to occlude the intestinal lumen. The bird harboring 6 worms, a female, died following a routine capture, examination, and collection of a blood sample. Possibly, this infection was a contributing factor to that death, the only capture-associated death during the study. The other dead bird, a second-year male, was found partially intact. We can only assume that the observed condition of the body was due to predation or postmortem actions of a scavenger. The intensities of worms found in the two dead birds are comparable to those found in dead western bluebirds, namely an unspecified “large number” (Bildfell et al., 2001) and five (Thompson-Cowley et al., 1979).

Cestoda
***Hymenolepis* sp.**
Weinland, 1858

Prevalence: One of 19 adult birds (5.2%) we examined by necropsy was infected. Five of 189 fecal samples (2.6%), collected during live examinations and examined using the flotation technique, contained ova. Identification was based on morphologic descriptions matching that of *Hymenolepis* ova.

Site of infection: Intestinal tract.

Remarks: The single specimen we collected during necropsy of a sacrificed bird was not retained because of its poor condition. We found no published reports of *Hymenolepis* sp. in eastern bluebirds; however, E. E. Wehr collected a cestode identified as *Hymenolepis* sp. from a “bluebird” from Falls Church, Virginia, U.S.A., in 1934 (USNPC 032770).

Nematoda
Capillarid nematode

Prevalence: Seven of 189 tested fecal samples (3.7%) from adult birds contained ova.

Site of infection: Intestinal tract.

Remarks: We based this general identification on the characteristic morphology of the ova, which are not diagnostic beyond the family level in capillarids. Although one capillarid, *Pterothomix exilis*, was reported in a captive eastern bluebird in the United Kingdom (Baruš and Sergejeva, 1990), we found no other reports of capillarids in eastern bluebirds. Our discovery presumably represents a new host record for the unidentified capillarid.

Spiruroid nematode

Prevalence: One of 189 tested fecal samples (0.5%) contained ova.

Site of infection: Intestinal tract.

Remarks: We based this identification on the presence of larvated ova characteristic of most spiruroid nematodes. Two spirurids, *Dispharynx nasuta* (Wehr, 1971) and *Oxyspirura pusillae* (Pence, 1972), were previously reported in eastern bluebirds; however, those accounts were based on the collection of adult worms rather than the ova. Because definitive identification is not possible using ova alone, we cannot say whether this report represents a new host record or an additional record of a previously reported species.

Strongyloid nematode

Prevalence: Seven of 189 tested fecal samples (3.7%) contained ova.

Site of infection: Intestinal tract.

Remarks: We used morphologic characteristics common to ova of strongyloid nematodes to make this general identification. Because there are no previous accounts describing adult strongyloids or ova from eastern bluebirds, we believe this constitutes a new host record.

Arthropoda
Carnus floridensis
Grimaldi, 1997

Prevalence: One of 103 examined clutches (1.0%) was infested.

Site of infestation: Integument.

Specimens deposited: USDA (NVSL 497050).

Remarks: Adult, nestling bird flies (*C. floridensis*), one of the previously unreported macroparasites of eastern bluebirds, were found in one nest. This blood-sucking fly is 1 of 4 described species (25%) in the genus occurring in North America (Grimaldi, 1997). Adults parasitize nestlings of wild birds, and larvae live in the nest materials, probably as scavengers. The only previously known specimens of *C. floridensis* are those in the type series from an undesignated woodpecker nestling in Florida, U.S.A. (Grimaldi, 1997), and two other Florida collections, both from nestling great-crested flycatchers (*Myiarchus crinitus*) (NVSL 98-7999, 98-8001) (Forrester and Spalding, 2003). Bequaert (1942) reported one other collection of a *Carnus* sp. fly (as *C. hemipterus*) from

Table 3. Parasitic arthropods previously detected on eastern bluebirds (*Sialia sialis*) based on literature reports (1908–2009).*

Arthropod	Host locality†	Reference
Ticks		
<i>Amblyomma americanum</i>	GA	Mataxas and Pung (1999), Durden et al. (2001), Wilson and Durden (2003)
<i>Amblyomma maculatum</i>	GA	Durden et al. (2001), Wilson and Durden (2003)
	ns	Bishopp and Trembley (1945)
<i>Haemaphysalis leporispalustris</i>	IA	Joyce and Eddy (1943)
	ns	Bishopp and Trembley (1945)
<i>Ixodes brunneus</i>	GA, TN	Pitts and Hays (1990), Luttrell et al. (1996)
Feather mites		
<i>Amerodectes sialiarum</i>	NC	Reeves et al. (2007), Carleton and Proctor (2010)
	GA, Guatemala	Valim and Hernandez (2008)
<i>Analgēs</i> sp.	GA	Carleton and Proctor (2010)
<i>Analgopsis</i> sp.	NC	Peters (1936)
<i>Mesalgoides</i> sp.	NC, GA	Reeves et al. (2007), Carleton and Proctor (2010)
<i>Proctophyllodes vesca</i>	TX	Atyeo and Braasch (1966)
<i>Pterodectes</i> sp.	Guatemala	Valim and Hernandez (2008)
<i>Trouessartia</i> sp.	NC	Reeves et al. (2007)
<i>Trouessartia sialiae</i>	GA	Carleton and Proctor (2010)
Skin mites		
<i>Ornithocheyletia canadensis</i>	ONT, Canada	Banks (1909)
Chigger mites		
<i>Eutrombicula cinnabaris</i>	KS	Wolfenbarger (1952), Loomis (1956)
<i>Eutrombicula lipovskiana</i>	KS	Wolfenbarger (1952), Loomis (1956)
<i>Neoschoengastia americana</i>	KS	Loomis (1956)
Nest mites		
<i>Dermanyssus hirundinis</i>	OH	Chow et al. (1983), Burt et al. (1991)
<i>Dermanyssus prognepphilus</i>	SC	Peters (1936), Moss et al. (1970)
	GA	Mataxas and Pung (1999)
	NC	Reeves et al. (2007)
<i>Ornithonyssus sylviarum</i>	FL	Neece (1990)
Nasal mites		
<i>Boydia spatulata</i>	LA	Pence (1973)
<i>Sternostoma sialiphilus</i>	LA	Pence (1973)
	MI	Hyland and Ford (1961)
Fleas		
<i>Ceratophyllus</i> sp.	NY	Dobrosky (1925)
<i>Ceratophyllus diffinis</i>	NH	Jordan (1929)
	ns	Fox (1940), Benton and Shatrau (1965)
<i>Ceratophyllus gallinae</i>	CT	Fuller (1943)
	MA	Jordan (1928), Fuller (1943)
	NC	Nelder et al. (2005)
	WV	Eckerlin and Painter (2000)
	ns	Boyd (1951)
<i>Ceratophyllus idius</i>	MA	Jordan (1928)
	NE	Main (1970), Fuller (1943)
	ns	Fox (1940), Lewis and Galloway (2001)
<i>Ceratophyllus niger</i>	ns	Fox (1940)
<i>Ceratophyllus petrochelidoni</i> ‡	ns	Lewis and Galloway (2001)
Lice		
<i>Philopterus sialii</i>	FL	Peters (1936), Forrester and Spalding (2003)
	GA	Wilson and Durden (2003)
	NC	Peters (1936), Reeves et al. (2007)
	NH, NY, OH, SC, VA	Peters (1936)
	TN	Reeves et al. (2007)
	ns	Malcomson (1960), Price et al. (2003)

Table 3. Continued.

Arthropod	Host locality†	Reference
<i>Ricinus</i> sp.‡	FL	Peters (1936)
Hippoboscid flies		
<i>Ornithomyia anchineuria</i>	MA NH QUE, Canada	Bequaert (1954), Main and Anderson (1970) Peters (1936) Savard (1996)
Myiasis flies		
<i>Phaenicia coeruleiviridis</i>	FL	Spalding et al. (2002)
<i>Philornis porteri</i>	FL	Spalding et al. (2002)
<i>Protocalliphora</i> sp.	MA MI MN NH ns NY OH ONT, Canada RI VA	Mason (1936, 1944) Pinkowski (1977) Berner et al. (1992) Shelley (1934) Herman (1936) Dobrosky (1925), Berner and Mallette (1993) Chow et al. (1983) Krug (1941) Berner and Mallette (1993) Campbell (1982)
<i>P. asiovora</i> ‡	ns	Whitworth (2003)
<i>P. braueri</i>	ns	Whitworth (2003)
<i>P. deceptor</i>	TX	Whitworth (2003)
<i>P. hirundo</i>	MA	Sabrosky et al. (1989)
<i>P. shannoni</i>	MA	Sabrosky et al. (1989)
<i>P. sialia</i>	CT KY IN, MN, NJ, NY, OH, TN, VA, WV, WI MA MI NC NY ONT, Canada PA QUE, Canada ns	Wetherbee (1932) Davis et al. (1994) Whitworth (2003) Henshaw (1908) Kenaga (1961), Sabrosky et al. (1989) Reeves et al. (2007) Roby et al. (1992), Wittmann and Beason (1992) Darling and Thomson-Delaney (1993) Miller (1909), Hannam (2006) Lloyd (1922) Rogers et al. (1991)
<i>Synthesiomyia nudiseta</i>	FL	Spalding et al. (2002)
Biting flies		
Simuliidae	AR	National Wildlife Health Center (2008)

*GA, Georgia; ns, not specified; IA, Iowa; TN, Tennessee; NC, North Carolina; TX, Texas; ONT, Ontario; KS, Kansas; OH, Ohio; SC, South Carolina; FL, Florida; LA, Louisiana; MI, Michigan; NY, New York; NH, New Hampshire; CT, Connecticut; MA, Massachusetts; WV, West Virginia; NE, New England; VA, Virginia; QUE, Quebec; MN, Minnesota; RI, Rhode Island; KY, Kentucky; IN, Indiana; NJ, New Jersey; WI, Wisconsin; PA, Pennsylvania; AR, Arkansas.

†Localities are within the United States unless specified otherwise.

‡Species not specified.

Florida screech-owl nestlings (*Megascops asio*) that probably was *C. floridensis* (Grimaldi, 1997; Forrester and Spalding, 2003). Ours is the first collection of this fly from outside of Florida.

***Dermanyssus prognephilus* Ewing, 1922**

Prevalence: Forty-five of the 103 nests (43.7%) we examined were infested with mites.

Site of infection: Nesting material and integument of birds.

Specimens deposited: U.S. National Parasite Collection (USNPC 102322.00).

Remarks: Commonly known as the martin mite, *D. prognephilus* has been reported in not only purple martin nests (Peters, 1936) but also in the nests of many species of cavity-nesting birds (Moss et al.,

1970). We routinely observed blood-engorged mites crawling on nestlings while banding them, and in a single nest, we counted 28,563 individuals. We did not quantify the mites from every nest examined or every nestling with mites. Its known geographic distribution (Moss et al., 1970) and information presented in Table 3 hints at the presumably widespread, common occurrence of *Dermanyssus* spp. mites elsewhere in conjunction with bluebird nests.

***Campephiloptes* sp.
Fain et al., 1982**

Prevalence: One of 103 nests (1.0%) examined was infested with this mite.

Site of infestation: Nestling material.

Specimens deposited: USDA (NVSL 505963).

Remarks: We identified 2 female individuals from one of the examined nests. These mites (Astigmatina: Pyroglyphidae) are from a group of typically nidicolous species that also includes many kinds of house dust mites. Most of the mites in this family are detritivores in bird nests, but some kinds are true feather mites, living in the plumage. A few species function as both detritivores and as feather mites. Because the only 2 known species of *Campephiloptes* are associated with large woodpeckers in Paraguay and Venezuela (Fain et al., 1982; Colloff, 2009), the mites we collected are new geographic and host records and probably are a previously unknown species. Whether these mites were functioning as detritivores or true feather mites is unknown because we elected not to sacrifice nestlings to collect ectosymbionts by the body washing method.

***Ixodes brunneus*
Koch, 1844**

Prevalence: One of the 19 birds (5.2%) we examined by necropsy was tick infested, and only 1 of the 230 birds (0.4%) we captured and examined live was infested. Each bird harbored a single tick.

Site of infestation: Integument near aural canal in both cases.

Specimen deposited: SCWDS, University of Georgia, Athens, Georgia, U.S.A. No accession number.

Remarks: One specimen of *Ixodes brunneus* collected during our study was plausibly responsible for the death of an adult female discovered dead within an empty nest box. This death occurred very early in

the breeding season, which coincides with the highest recorded seasonal occurrences of this tick species (Luttrell et al., 1996). The female, which harbored a single tick, was extremely emaciated and presumably died of starvation secondary to tick paralysis. The body had no signs of trauma, and the overnight temperatures in the week preceding the bird's discovery had been mild. There have been several reports of tick paralysis associated with attachment of a single tick not only in bluebirds (Luttrell et al., 1996) but also in a snake (*Coluber constrictor priapus*) (Hanson et al., 2007) and in humans (Grattan-Smith et al., 1997).

***Philoaterus sialii*
Osborn, 1896**

Prevalence: Four of 14 birds (28.6%) were infested. We collected lice from 4 of the 230 birds (1.7%) that were captured and examined.

Site of infestation: Integument (plumage).

Specimens deposited: U.S. National Parasite Collection (USNPC 102323.00).

Remarks: This host-specific chewing louse is the only louse species associated with eastern bluebirds (Price et al. 2003).

Based on results of our historical review of literature and online search for collection records, 2 viral infections and at least 6 species of hematozoa have been reported previously from eastern bluebirds (Table 2). In addition, macroparasites previously reported from eastern bluebirds include 3 identified species of nematodes, 5 reports of unidentified microfilaria, 1 species of cestode, 2 species of trematodes (Table 4), 4 tick species, 3 species of nest mites, 2 species of nasal mites, 8 species of feather mites, 1 species of skin mite, 3 species of chigger mites, 5 to 6 species of fleas, 1 to 2 species of lice, 1 species of hippoboscid fly, 10 to 11 species of myiasis flies, and 1 species of biting fly (Table 3).

DISCUSSION

Up to 10 of the species of parasites we collected have not been reported previously in eastern bluebirds (i.e., *Atoxoplasma* sp., *P. relictum*, *Sarcocystis* sp., *C. floridensis*, *Campephiloptes* sp., *P. cylindraceus*, *Hymenolepis* sp., a capillarid nematode, a strongyloid nematode, and a spiruroid nematode). With the exception of *I. brunneus* and *P. cylindraceus*, the adverse consequences of parasitism on individual birds we observed seemed to be minimal.

Table 4. Helminths previously detected in eastern bluebirds (*Sialia sialis*) based on literature reports (1935–2009).*

Parasite	Location in situ	Host locality†	Reference
Nematoda			
<i>Dispharynx nasuta</i>	ventriculus	ns	Wehr (1971)
<i>Oxyspirura pusillae</i>	eye	LA	Pence (1972)
Microfilaria‡	blood	GA	Love et al. (1953), Mataxas and Pung (1999)
	blood	MD, NJ	Williams and Bennett (1978)
	blood	ns	Greiner et al. (1975)
	lung	ns	Robinson (1954)
<i>Pterothominx exilis</i>	intestine	United Kingdom (ns, captive)	Baruš and Sergejeva (1990)
Trematodes			
<i>Lutztrema monenteron</i>	gall bladder	GA	Krissinger (1984)
		VA	Price and McIntosh (1935)
<i>Collyriclum faba</i>	subcutis	NY	Kibler (1968)
		MI	Pinkowski (1975)

*ns, not specified; LA, Louisiana; GA, Georgia; MD, Maryland; NJ, New Jersey; VA, Virginia; NY = New York.

†Localities are within the United States unless specified otherwise.

‡Species not specified.

Any indirect negative effects on reproduction and long-term survival are unknown; however, concurrent infections/infestations or those in combination with physiologic stress factors have been reported to reduce host survival during severe weather conditions or to reduce overall fecundity in other bird species (Hudson, 1986; Chapman and George, 1991).

Microparasites

We detected 5 species of protistan parasites and 2 viruses within the bluebird population under study. Two of the microparasites, *Atoxoplasma* sp. and *Sarcocystis* sp., represent new host–parasite associations, and *P. relictum* potentially represents a third new association.

The prevalences of *Atoxoplasma* sp., *Sarcocystis* sp., and *Plasmodium* sp. reported here are based on small sample sizes and may not accurately reflect the actual prevalence within the population. Despite the high prevalence (71.4%) of *P. relictum* infections detected by PCR, none were detected in blood smears from the larger sample of live birds. This observation is not unexpected because others have shown chronic infections by *Plasmodium* spp. are very difficult to detect by the latter method when compared with PCR methods (Atkinson and van Riper, 1991; Waldenström et al., 2004).

Macroparasites

Overall prevalence of macroparasites within the studied adult bluebird population was low (16.1%)

compared with the prevalence of microparasites. However, this figure is probably an underestimate because of the modest number of necropsies we performed.

The numbers of arthropods we found by the live examination method were extremely low compared with numbers collected by body washing before necropsy. For comparison, Walther and Clayton (1997) detected fewer lice on rock doves (*Columba livia*) by visual examination than by fumigation. Although the dust-ruffling method that incorporates a pyrethrin-based insecticide enhances the collection of ectoparasites from live birds, we elected not to use it because of increased handling time (Walther and Clayton, 1997). In another study, body washing removed greater than 95% of resident lice from house sparrows (*Passer domesticus*) (McGroarty and Dobson, 1974). Assuming similar success with bluebird body washing, our estimated prevalence of *P. sialis* (28.5%) is probably representative of this population.

Macroparasite prevalence among observed nestlings was lower than that found in other studies of eastern bluebird nestlings (Mason, 1944; Pinkowski, 1977; Chow et al., 1983). Fewer than 50% of the nests we examined were infested with the hematophagous nest mite, *D. prognepphilus*. This is less than the prevalence of *D. hirundinis* (100.0%) among eastern bluebird nests examined in Ohio, U.S.A. (Chow et al., 1983) or the estimated 98.0% infested by *D. prognepphilus* in southeastern Georgia, U.S.A. (Mataxas and Pung, 1999).

Our study expands the known number of parasite species associated with eastern bluebirds to 68

species. Although the checklist we assembled is probably not all-inclusive, we compiled documentation of eastern bluebird parasites that predate records listed by Roberts (1981) by 26 years, and we added more recent reports published between 1977 and 2010. The list includes 81 reports from 27 states in the United States, 2 Canadian provinces, Guatemala, and the United Kingdom. Our list embraces at least 30 additional species, including the 5 to 10 previously undocumented species we detected during our field study. This work should provide both greater information and a time-savings for others investigating host–parasite relationships involving eastern bluebirds and possibly other species of passerines. We believe further investigation of these relationships in seemingly well-studied species could yield new and valuable information, and we encourage researchers of eastern bluebirds and other species to broaden the scope of their investigations by employing multiple parasite-collection methods, including the refinement of existing techniques and application of new ones.

ACKNOWLEDGMENTS

This study was supported in part by mentor support funding from the National Science Foundation Research Experience for Undergraduates and an H. Branch Howe, Jr., Research Grant provided by the Georgia Ornithological Society. Research results from the years 2004 through 2006 reported here were part of a dissertation submitted by R.E.C. in partial fulfillment of the requirements for a Ph.D. degree at the University of Georgia, Athens, Georgia, U.S.A. We thank Berry College, Mount Berry, Georgia, U.S.A., for access to the study areas; A. Watson, B. Daniels, H. Pruett, J. Christian, and G. Hightower for their assistance in monitoring nest boxes and nest examinations. All sample collections and animal procedures were carried out under approval of the Institutional Animal Care and Use Committee of Berry College and federal and state scientific collection permits. Dr. Andrew Allison and staff members of the Southeastern Cooperative Wildlife Disease Study graciously assisted in parasite identification, plaque reduction neutralization testing, and PCR procedures. We also thank Dr. Heather Proctor for her assistance with nest mite identification. Able library assistance was provided by J. Alfred and J. Eifling. Deepest gratitude is also extended to S. Schweitzer and 2 anonymous reviewers for suggestions regarding this manuscript.

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